# A photofabricated honeycomb micropillar array for loss-free trapping of microfluidic droplets and application to digital PCR

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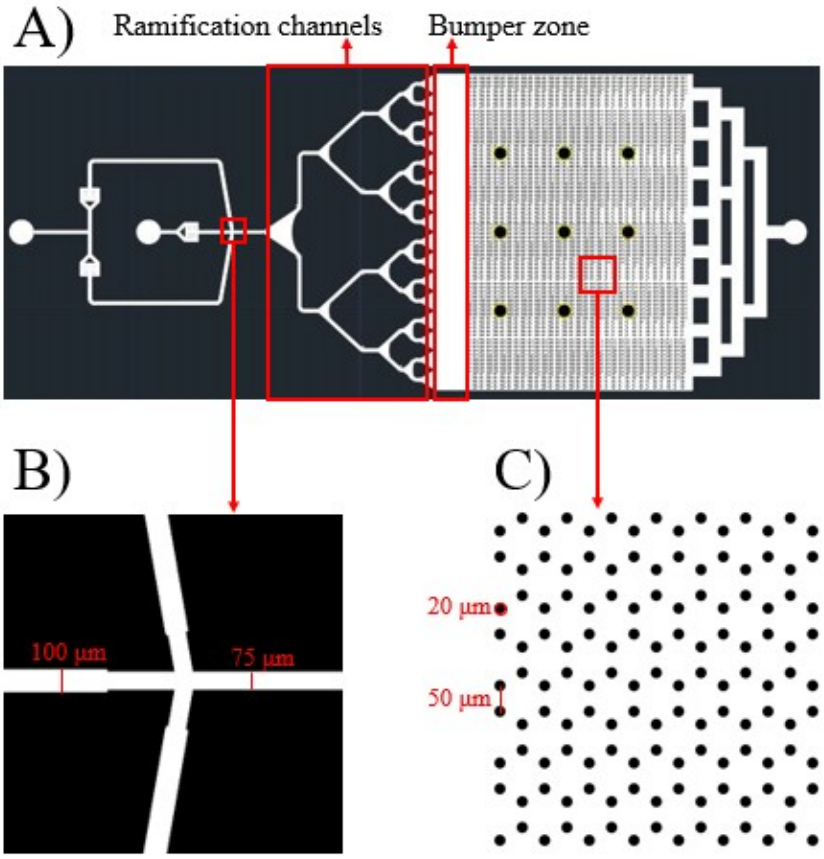


Fig. S1 A) Detailed channel structure of the photofabricated honeycomb micropillar array chip. B) The dimension of flow-focusing droplet generator is 75×75 µm. C) The micropillars with 20 µm diameters are arranged in a regular hexagonal pattern to form a honeycomb-like array. The channel depth is 50 µm.

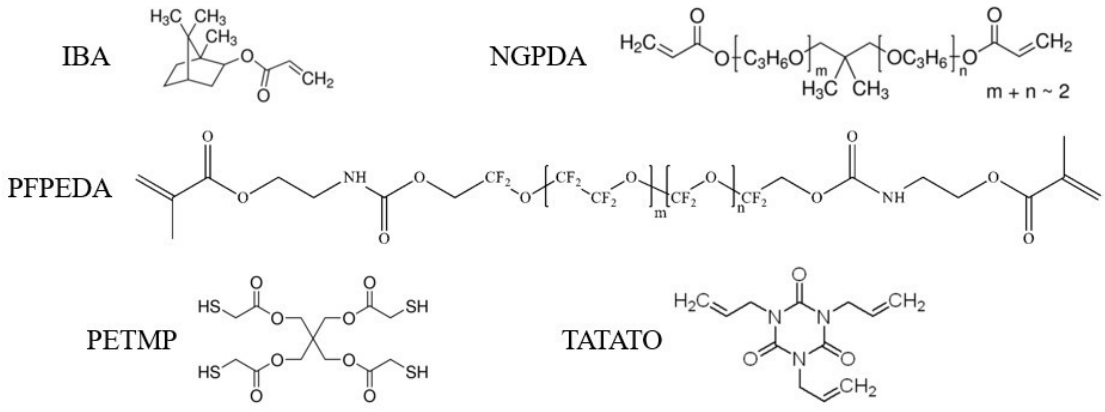


Fig. S2 Chemical structures of the photocurable materials used for chip fabrication, including isobornyl acrylate (IBA), neopentyl glycol propoxylate diacrylate (NGPDA), perfluoropolyether dimethacrylate (PFPEDA), pentaerythritol tetrakis(mercaptoacetate) (PETMP), triallyl-1,3,5-triazine-2,4,6(1H,3H,5H)-trione (TATATO). Note: The chemical structure of polyurethane acrylate (PUA, 6115J-80, Eternal Materials Co., Ltd) is unknown.

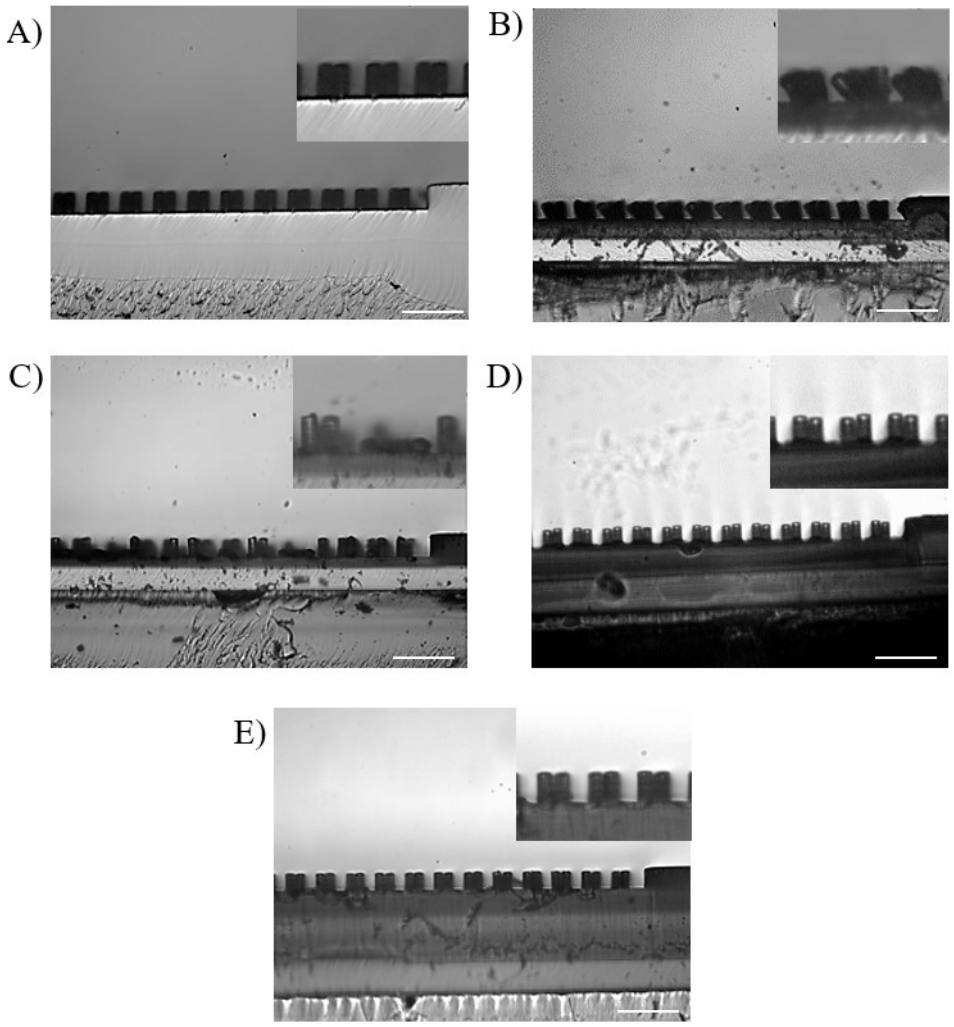


Fig. S3 Cross-section view of the micropillar arrays made of A) PDMS, B) IBA, C) NGPDA, D) PFPEDA, and E) PUA, respectively. Inset on the top right shows an enlarged view. Scale bar: 200 μm.

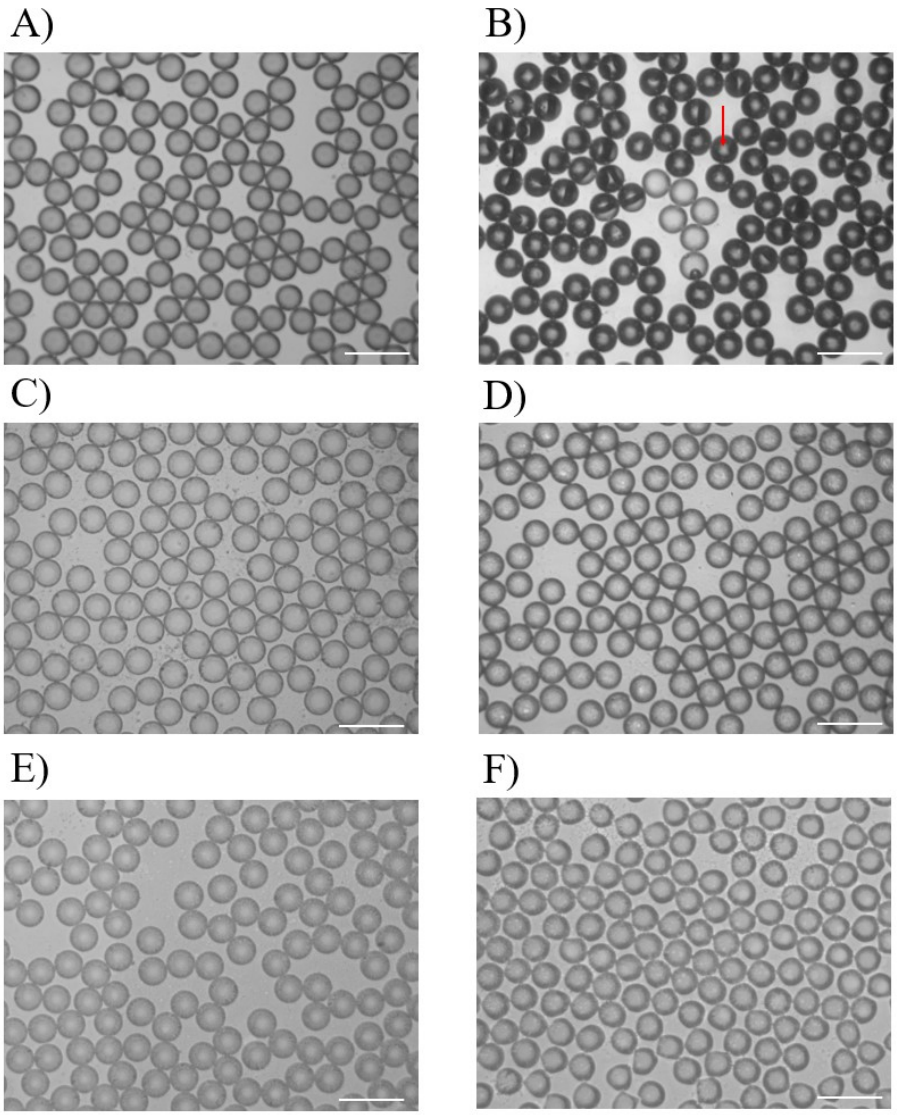


Fig. S4 Comparison of PUA, TE, and PFPEDA-based chips for anti-evaporation. Herein, the water-in-oil droplets were collected and UV-cured inside a large chamber made of A) PUA, C) TE, and E) PFPEDA respectively, and then subjected to PCR thermocycling. Results showed that the droplets in B) PUA chip undergo serious evaporation, leaving a lot of empty cavities (as indicated by the red arrow) after thermocycling, while no droplet evaporation occurred in the D) TE and F) PFPEDA devices under the identical conditions. Scale bar: 200 μm.

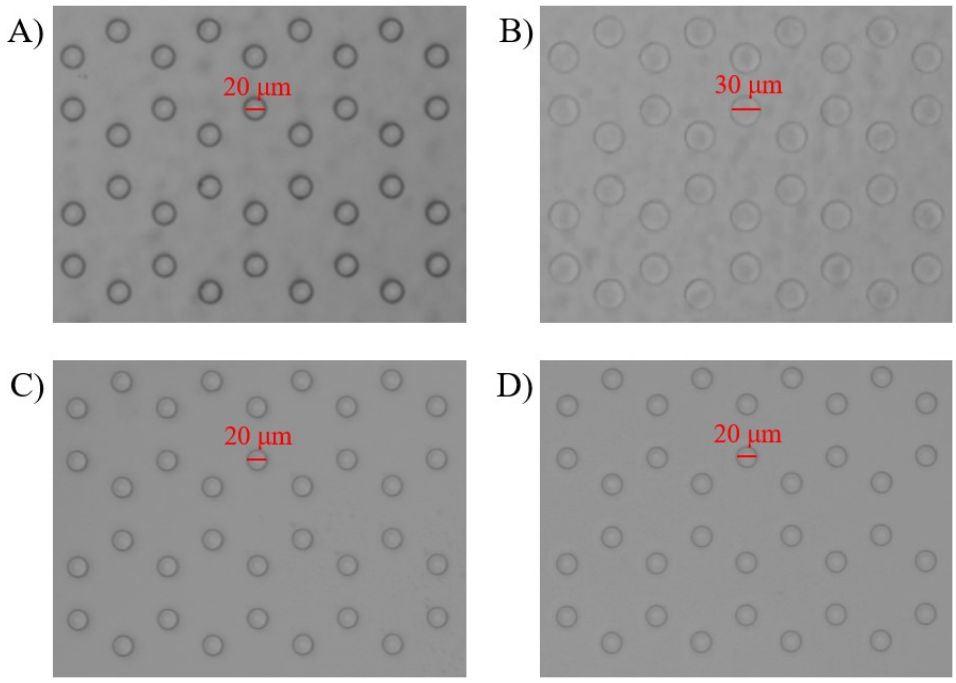


Fig. S5 Microscopic images of PUA and PFPEDA-based micropillar arrays A) C) before and B) D) after treated with the photocurable oil.

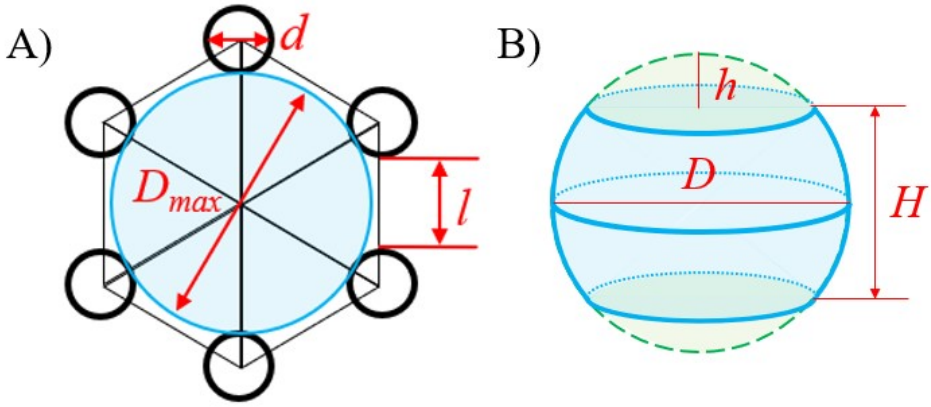


Fig. S6 A) Schematic description of one trapping unit. Herein, *d* is the diameter of micropillar, *l* is the spacing between adjacent micropillars. B) Schematic of the squeezed droplet (the blue part), which can be considered as a sphere with two spherical crowns (the green part) being cut off. *H* and *h* are the heights of the chamber and the spherical crown, respectively.

The maximal droplet that can be trapped is relatively easy to estimate, whose diameter $D_{max}$ should not exceed the internally tangent circle of the trapping unit.

$$D_{max} = 2l + d \tag{1}$$

Since the large droplet would be squeezed into the drum-like structure in the device, we herein use the circle diameter at the vertical center (as shown in Fig. S6B) to define its size. To estimate its volume, we assume that it is the remaining part of a sphere when two spherical crowns are cut off symmetrically. Accordingly, the droplet volume $V_d$ can be described as:

$$V_d = V_s - 2V_c \tag{2}$$

Wherein, $V_s$ is the volume of the sphere, and $V_c$ is the volume of each spherical crown.

$$V_s = \frac{4\pi}{3}\left(\frac{D}{2}\right)^3 \tag{3}$$

$$V_c = \frac{\pi}{3}h^2\left(3 \times \frac{D}{2} - h\right) \tag{4}$$

As a result, the volume of maximal droplet can be calculated according to the following equation:

$$V_{d\text{-}max} = \frac{4\pi}{3}\left(\frac{D_{max}}{2}\right)^3 - 2 \times \frac{\pi}{3}h^2\left(3 \times \frac{D_{max}}{2} - h\right) \tag{5}$$

Where $h = (D_{max}\text{-}H)/2$ is the height of the spherical crown. Then the above formula can be simplified to:

$$V_{d\text{-}max} = \frac{\pi}{12}\left(3D_{max}^{2}H - H^{3}\right) \tag{6}$$

In order to make the trapping unit unable to accommodate two droplets, one can easily to think that their total volume should be equal to or larger than that of the maximal droplet. Therefore, the minimal droplet volume can be considered as:

$$V_{d\text{-}min} = V_{d\text{-}max}/2 \tag{7}$$

To calculate its diameter, one should consider the following two circumstances.

1) When the minimal droplet diameter is larger than the chamber height, i.e., $D_{min} > H$, its volume can be calculated using the identical procedure mentioned above.

$$V_{d\text{-}min} = \frac{\pi}{12}\left(3D_{min}^{2}H - H^{3}\right) \tag{8}$$

Then, according to formula (1), (6), (7), and (8), the minimal droplet diameter can be estimated as:

$$D_{min}^{2} = \frac{3(2l+d)^{2} + H^{2}}{6} \tag{9}$$

2) When the minimal droplet diameter is smaller than or equal to the chamber height, i.e., $D_{min} \leq H$, its volume should be calculated corresponding a sphere. At this time, the minimal droplet diameter can be estimated as:

$$D_{min}^{3} = \frac{3(2l+d)^{2}H - H^{3}}{4} \tag{10}$$

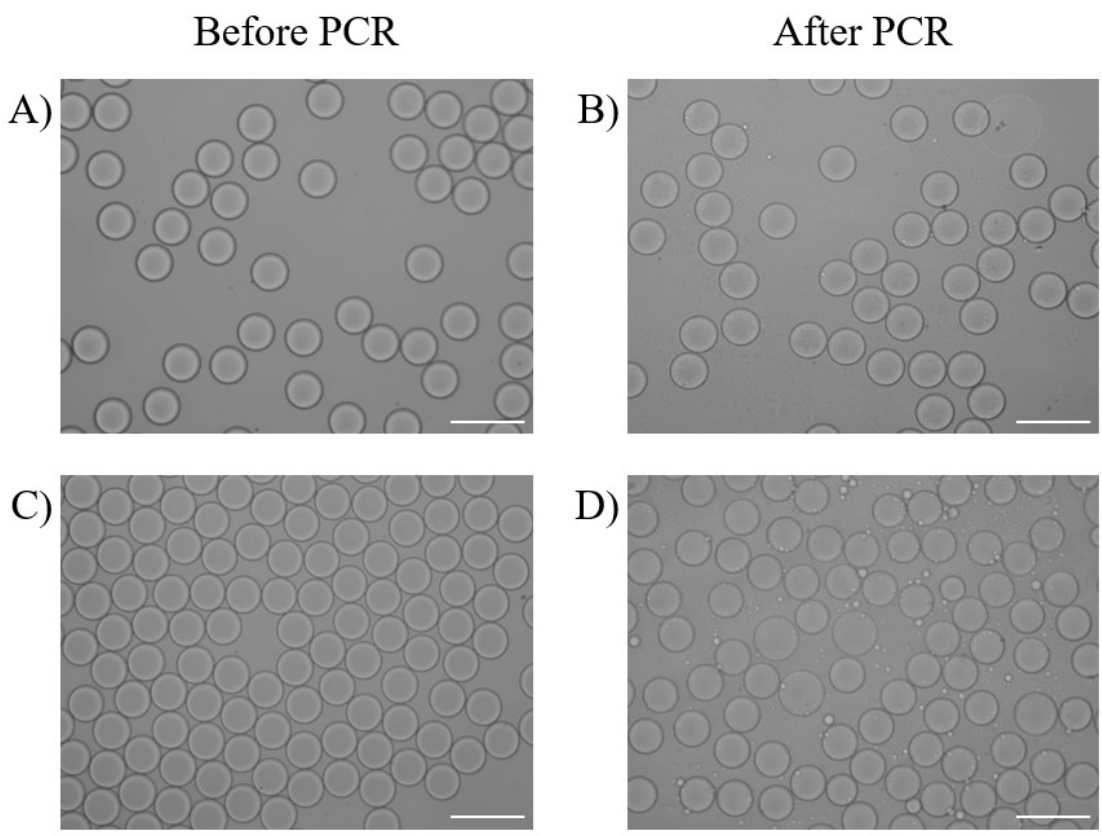


Fig. S7 Fluorinated oil HEF 7500-based droplets before and after PCR thermocycling in a tube (A, B) and a 50 µm deep chamber (C, D), respectively. Scale bar: 200 µm.

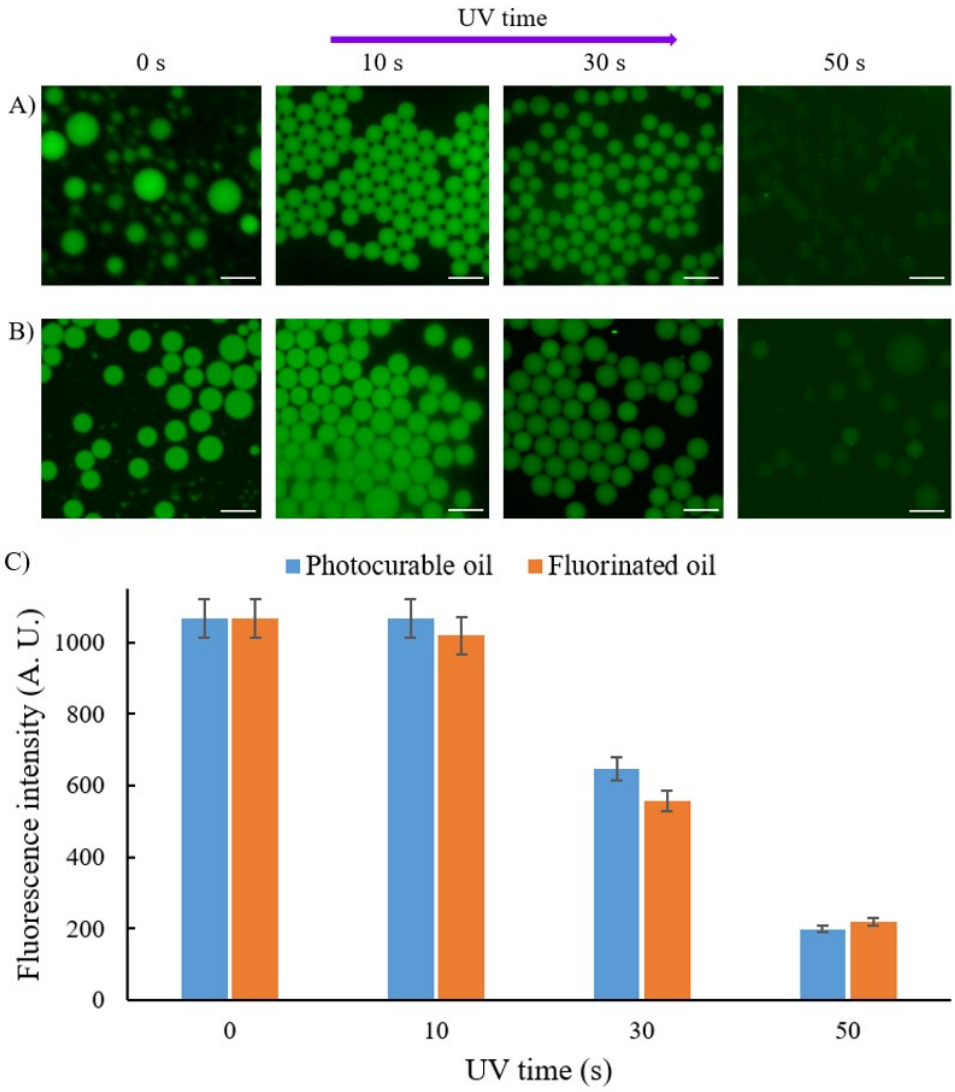


Fig. S8 Effects of UV irradiation (365 nm, 69 mW/cm$^2$) on PCR reaction in A) photocurable oil and B) fluorinated oil-based droplets. C) Fluorescence intensity extracted from a circle area at the center of each droplet. The results show that UV irradiation less than 10s (690 mJ/cm$^2$) has nearly no influence on PCR reaction. However, further extending the exposure time will notably decrease the fluorescence intensity, probably because of the possible damage to DNA and polymerase caused by reactive oxygen species, or the quenching of fluorescent dyes.

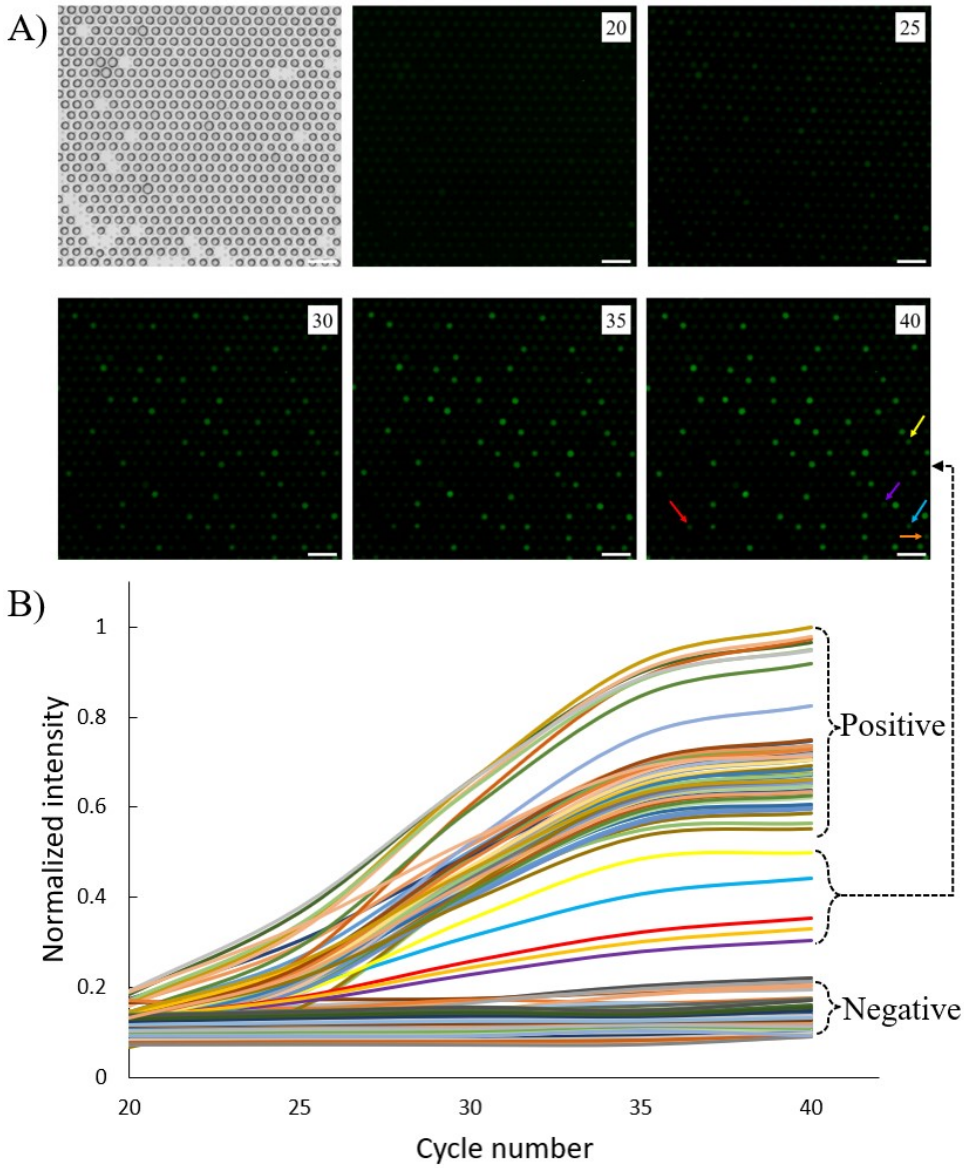


Fig. S9 A) The bright field image of droplets before PCR thermocycling and the fluorescence images recorded between 20$^{th}$-40$^{th}$ cycles with five-cycle increments. Scale bar: 200 μm. B) Normalized fluorescence intensity extracted from 200 droplets against PCR cycle numbers. The arrows in the image of the 40$^{th}$ cycle indicate the droplets with relatively weak fluorescence, which are in the same colors corresponding to the fluorescence curves.

Table S1 The UV exposure time for different photocurable materials used in PHMA photofabrication.

| Photocurable materials | Exposure time [a] (s) | | |
|---|---|---|---|
| | Top layer | Bottom sheet | Bonding |
| IBA | 100 | 60 | 200 |
| NGPDA | 100 | 60 | 200 |
| PFPEDA | 220 | 100 | 200 [b] |
| PUA | 220 | 100 | 200 |
| TE | 40 | 20 | 100 |

[a] UV irradiation: 365 nm, 2.5 mW/cm$^2$.

[b] Performed in nitrogen environment.

**Movies:**

Movie S1 Droplets running away from the chamber without micropillar array

Movie S2 Fluorinated oil-based droplets can hardly enter micropillar array (5x frame rate increase)

Movie S3 Complete process showing the generation and loss-free trapping of photocurable oil-based droplets